NERL Research Abstract

EPA's National Exposure Research Laboratory
GPRA Goal 2 - Clean and Safe Water

Significant Research Findings

Evaluation of Method 1622 for *Cryptosporidium* for Use in the Information Collection Rule

Purpose

Recent evidence regarding the safety of drinking water indicates that established and emerging pathogens continue to pose a human health risk. Outbreaks of disease linked to drinking water have been described for *Cryptosporidium*. This microbe causes gastroenteritis of varying severity and sometimes leads to death. One such occurrence was an outbreak in Milwaukee, Wisconsin in 1993 that sickened over 400,000 people and resulted in over 100 deaths. Large outbreaks of waterborne disease lend themselves to observation and study because of their size. However, many other cases of illness undoubtably occur each year, but are either unrecognized or under reported. The number of these cases may be significant, but little is known about the extent of this problem.

To characterize risks from drinking water pathogens, three essential needs must be met. First, methods for detection and measurement must be available. Second, it must be determined if these pathogens occur in source or finished drinking water and that people are exposed. Third, it must be shown that the microbes are capable of overwhelming human natural defense barriers and cause infection in the exposed population.

The American Society of Testing and Materials's Method P229 and Standard Methods' technique for detecting *Cryptosporidium* in source or drinking water are essentially the same. However, this approach has poor precision and specificity for estimating the occurrence of pathogens in source and drinking water. Therefore, the purpose of this project is to improve the method for detecting and *Cryptosporidium* oocysts in water samples with currently available technology.

Research Approach

This project focused on improving four areas of the existing methods: 1) changing from a nominal to an absolute porosity filter for sampling, 2) reducing the amount of water needed for sampling, 3) concentrating the parasites by immunomagnetic separation, and 4) counter staining with 4",6-diamidino-2-phenylindole (DAPI).

Major Findings and Significance

Around 60 percent of the *Cryptosporidium* oocysts pass through a 1.0 µm nominal porosity filter. In contrast, using a 1.0 µm absolute porosity filter, greater than 90 percent of the seeded oocysts are recovered. A 10 liter sample is adequate for Method 1622 and has reduced the processing required by other methods. Immunomagnetic separation is highly successful in concentrating *Cryptosporidium* away from other particulates in a water sample. Consequently, the time required for microscopic results is greatly reduced. Counter staining with DAPI allows sporozoite nuclei in some oocysts to be demonstrated more often than with differential contrast microscopy alone. Overall comparison of Method 1622 with previous methods showed that oocyst recoveries were 3 times better. This is particularly noteworthy, because oocyst seeding was done with around 100 oocysts, a number relevant to environmental oocyst densities, rather than the thousands required for the previous methods. The variability encountered with Method 1622 was half that associated with the previous methods. Based on the higher recoveries using much lower oocyst seeds in the validation study, Method 1622 is much more sensitive than the previous methods.

A study done in conjunction with the U.S. Geological Survey evaluated the performance of Method 1622. The results demonstrate that *Cryptosporidium* oocysts can be recovered from stream waters using Method 1622, but oocyst recoveries are lower, by 12 to 22 percent, than from seeded reagent-grade water (39-47 percent, from high purity water produced in the laboratory). Of the natural water samples that did not have parasites added to them, few had positive results.

Research Collaboration and Publications

The evaluation of filters used for Method 1622 was done in collaboration with Donna S. Francy of the U.S. Geological Survey, Columbus, OH 43229 (phone: 614-430-7769; e-mail: dsfrancy@usgs.gov).

Simmons, III, O.D., Heaney, C.D., Francy, D.S., Nally, R., Schaefer, III, F.W., Sobsey, M.D. Detection of *Cryptosporidium* oocysts in stream water samples using U.S. Environmental Protection Agency Method 1622. *Journal of the American Water Works Association*, 2000. In Press.

Simmons, III, O.D., Sobsey, M.D., Heaney, C.D., Schaefer, F.W., III, Francy, D.S. Concentration and detection of *Cryptosporidium* oocysts in surface water samples by Method 1622 using ultrafiltration and capsule filtration. *Applied and Environmental Microbiology*, 2000. Submitted.

Future Research

Future research calls for evaluation of Method 1623 in challenging natural water samples. Method 1623 is similar to Method 1622 but incorporates the pathogen *Giardia* into the analysis. Evaluation of an internal control for each individual analysis is planned as well. Once detection methods are fully

developed and validated, occurrence studies will be initiated.

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